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Adenosine Diphosphate-Induced Platelet-Fibrin Clot Strength: A New Thrombelastographic Indicator of Long-Term Post-Stenting Ischemic Events

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Abstract

Background—Post-stenting ischemic events occur despite dual antiplatelet therapy suggesting that a "one size fits all" antithrombotic strategy has significant limitations. *Ex vivo* platelet function measurements may facilitate risk stratification and personalized antiplatelet therapy.

Methods—We investigated the prognostic utility of the strength of ADP-induced (MA_{ADP}) and thrombin-induced ($MA_{THROMBIN}$) platelet-fibrin clots measured by thrombelastography and ADP-induced light transmittance aggregation (LTA_{ADP}) in 225 serial patients following elective stenting treated with aspirin and clopidogrel. Ischemic and bleeding events were assessed over three-years.

Results—Overall, 59 (26 %) first ischemic events occurred. Patients with ischemic events had higher MA_{ADP}, $MA_{THROMBIN}$, and LTA_{ADP} ($p<0.0001$ for all comparisons). By receiver operating characteristic curve analysis, MA_{ADP} > 47mm had the best predictive value of long-term ischemic events compared to other measurements ($p<0.0001$) with an area under the curve = 0.84 [95% CI 0.78 – 0.89, $p < 0.0001$]. The univariate Cox proportional hazards model identified MA_{ADP} >47mm, $MA_{THROMBIN}$ >69mm, and LTA_{ADP} >34% as significant independent predictors of first ischemic events at the three-year time point, with hazard ratios of 10.3 ($p<0.0001$), 3.8 ($p<0.0001$), and 4.8 ($p<0.0001$) respectively. Fifteen bleeding events occurred. Receiver operator characteristic curve and quartile analysis suggest $MA_{ADP} \leq 31$ as a predictive value for bleeding.

Conclusion—This study is the first demonstration of the prognostic utility of MA_{ADP} in predicting long term event occurrence following stenting. The quantitative assessment of ADP-stimulated platelet-fibrin clot strength measured by thrombelastography can serve as a future tool in investigations of personalized antiplatelet treatment designed to reduce ischemic events and bleeding.

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Disclosures

Eli Cohen and Irene Navickas are former employees of Haemoscope Corporation.

All other authors report no conflicts of interest.

INTRODUCTION

Ischemic events following percutaneous coronary intervention (PCI) are influenced by platelet activation and thrombin generation.[1,2] Antiplatelet therapy with clopidogrel and aspirin is the current standard of care for patients undergoing PCI. Despite the proven benefits of adding clopidogrel to aspirin therapy, a significant percentage of patients will experience both short and long term post-stenting ischemic events suggesting that the current "one size fits all" dosing strategy has significant limitations.3

The existing treatment paradigm of dual antiplatelet therapy is based on the results of prospective, randomized large scale clinical trials that did not include concomitant assessments of platelet function.[3] Currently, a uniform dosing and duration of antiplatelet treatment is recommended irrespective of the individual patient's response to antiplatelet therapy. The later approach is based on the inherent assumption that all post-stenting patients have the same level of antiplatelet response and hemostasis. The limitations of the latter approach have been revealed by demonstration of wide variability in clopidogrel responsiveness in multiple pharmacodynamic studies.[4] At one end of the spectrum, selected patients with excessively low on-treatment platelet reactivity may have unnecessary bleeding, whereas patients with high platelet reactivity may experience ischemic events. Subsequently, an increasing body of translational research data has demonstrated a strong relation of high on-treatment platelet reactivity primarily measured by conventional light transmittance aggregometry after stimulation by adenosine diphosphate (LTA_{ADP}) to post-stenting ischemic event occurrence.[4]

A previous report demonstrated that high thrombin-induced platelet-fibrin clot strength ($MA_{THROMBIN}$) measured by thrombelastography (TEG) and high LTA_{ADP} were risk factors for 6-month post- PCI ischemic events. In that study $MA_{THROMBIN}$ was a better risk discriminator.[5] Herein we report the results of a new long-term follow-up study where we measured ADP-induced platelet-fibrin clot strength (MA_{ADP}) by the TEG Platelet Mapping assay in addition to $MA_{THROMBIN}$ and LTA_{ADP} and compared their association with post-PCI ischemic event occurrence with the aim of future use of these tests to personalize antiplatelet therapy.

METHODS

Patients

The study was approved by the Investigational Review Board of Sinai Hospital, Baltimore, Maryland. Two hundred twenty-five consecutive patients undergoing non-emergent PCI were prospectively enrolled between 2004 and 2005 after providing informed consent. All patients were older than 18 years of age. Inclusion and exclusion criteria were described previously.[5] Aspirin (325 mg) was administered to all patients on the day of the procedure and daily thereafter (81-325 mg). Sixty-six percent of the patients received a 300 mg (n=73) or 600 mg (n=75) clopidogrel loading dose immediately following successful PCI, with a 75 mg/day maintenance dose thereafter. Patients on a maintenance dose of clopidogrel (75 mg/day) at the time of admission (n=77) did not receive a loading dose. Eptifibatide was administered to 123 patients. Unfractionated heparin was administered to all patients during the procedure to achieve a clotting time of 200-250 sec for patients administered a GPIIb/IIIa inhibitor, and >300 sec for all other patients.

Blood samples were obtained at 18-24 hours post-PCI; an additional sample was collected 5 days post-PCI in patients treated with eptifibatide. In those patients not treated with eptifibatide, measurements from the 18-24 hours sample were correlated with clinical outcomes, whereas in those patients treated with eptifibatide, the measurements from day 5 were used in the

analysis. In patients experiencing bleeding, an additional blood sample was obtained at or close to the time of the bleeding event. We collected three 5 mL vacutainer tubes (Beckton-Dickenson, Franklin Lakes, New Jersey), two containing 3.2% trisodium citrate for light transmittance aggregometry and one containing 40 USP lithium heparin for TEG PlateletMapping analysis.

Platelet –Fibrin Clot Strength Measurement

Platelet-fibrin clot strength measurements were carried out using the Thrombelastograph® (TEG® Hemostasis System, Haemoscope Corporation, Niles, IL). The TEG Hemostasis Analyzer with automated analytical software provides quantitative and qualitative measurements of the physical properties of a clot.[5]

The TEG technology is described elsewhere.[5,6] Briefly, a stationary pin is suspended into an oscillating cup that contains the whole blood sample. As the blood clots, it links the pin to the cup. Clot strength is determined by measuring the amplitude of the rotation of the pin, which increases proportionally with clot strength. Maximum amplitude represents maximum clot strength, expressed as the MA parameter.[5,6]

Light Transmittance Aggregation

Platelets in platelet rich plasma were stimulated with 5μM ADP, and 2mM arachidonic acid (AA) (Chronolog, Havertown, PA). Aggregation was assessed using a Chronolog Lumi-Aggregometer (Model 490-4D) with the Aggrolink software package. Aggregation was expressed as the maximum percent change in light transmittance, using PPP as a reference.[5,6]

Clinical Endpoints

Patients were followed for the occurrence of adverse events during index hospitalization and for up to 36 months. Patients were contacted either by phone or by office appointment to determine event occurrence and compliance with antiplatelet drug therapy. The primary endpoint was the composite of cardiac death, stent thrombosis, myocardial infarction, ischemic stroke, and unplanned revascularization. First events are reported. Cardiac death was defined as death secondary to any cardiovascular cause. Myocardial infarction was defined as the occurrence of symptoms of myocardial ischemia associated with troponin I values greater than upper limits of normal.[7] Stent thrombosis was defined as definite stent thrombosis according to the Academic Research Consortium.[8] The secondary endpoint was the composite of major and minor bleeding during the observation period. Bleeding was quantified according to the TIMI criteria.[9] Two independent physicians blinded to laboratory data adjudicated events after review of source documents.

Statistical Analysis

Categorical variables are expressed as n (%) and continuous variables as mean ± SD with $p<0.05$ considered statistically significant. The Fischer Exact test and Mann-Whitney Rank Sum test were used for comparison of categorical variables and Student's t-test was used for comparison of continuous variables between groups. Receiver operator characteristic curve (ROC) analysis (MedCalc software, Mariakerke, Belgium) was performed to identify the best discriminatory level of $MA_{THROMBIN}$, MA_{ADP}, and LTA_{ADP} associated with first ischemic events. The predictive values of the three measurements were assessed by ROC comparison analysis. To assess event-free survival, a Kaplan–Meier analysis was performed and the event–time data are reported in two curves according to the cutpoint of each platelet function parameter, determined by ROC analysis. Univariate comparisons between patients with and without ischemic events were performed using the chi-square test, Mann–Whitney U test, or

Student *t* test as appropriate. Demographic and procedural variables with *P* values below 0.1 in the univariate analysis were entered by stepwise forward selection in a multivariate Cox proportional hazards model (SAS software, Cary, NC).

Sample Size Calculation

A recent study demonstrated that ~20% of patients undergoing PCI will experience death or repeat revascularization within 3 years and it has been demonstrated that patients with high platelet reactivity have greater event frequency with adjusted hazard ratios reported at least $3.0.10^{-11}$ We hypothesized an ~ 25% incidence of ischemic events in patients with high platelet reactivity as compared to 10% incidence in patients with normal platelet reactivity. Using the sample size calculation from SigmaStat software (SigmaStat,Systat software, Inc., San Jose, CA), it was estimated that the sample size required for 90% power with the alpha of 0.05, and a 5% drop out was ~ 225 patients.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

RESULTS

Demographic and Procedural Characteristics

Two hundred twenty-five patients undergoing PCI were enrolled. Fifty-nine patients (26%) sustained an ischemic event within three years after the index PCI. Patient demographics and procedural characteristics are shown in Tables 1 and 2, respectively. Briefly, patients with ischemic events had a higher prevalence of hyperlipidemia, diabetes, prior PTCA, calcium channel blocker usage, and had lower ejection fraction as compared to patients without ischemic event. Patients within the ischemic group had more complex disease, smaller vessel diameter, and more implanted stents.

Ischemic Event Occurrence

Two percent of patients had an ischemic event within one month of PCI and 26 % experienced an ischemic event within the three years after the index PCI (Table 3). Twenty-four percent of patients (14/59) had their event after clopidogrel discontinuation with a mean duration of therapy of 6.4 ± 3 months. Ninety-eight percent of ischemic events occurred during aspirin treatment.

Association Between Platelet Function Parameters and Adverse Ischemic Events

In patients experiencing an ischemic event, measures of thrombin-induced and ADP-induced platelet-fibrin clot strength ($MA_{THROMBIN}$ and MA_{ADP}, respectively) were significantly higher than in patients free from ischemic events (Table 4). In contrast, AA-induced platelet-fibrin clot strength (MA_{AA}) was highly inhibited in both groups. The association of platelet function parameters and ischemic events was assessed with receiver operating characteristic (ROC) analysis as shown in Figure 1. In comparison with LTA_{ADP} and $MA_{THROMBIN}$, MA_{ADP} had the best predictive value yielding an AUC of 0.84 [95% CI (0.78 – 0.89), $p < 0.001$ for all comparisons].

In univariate Cox proportional hazard model analysis using the ROC cut point, $MA_{THROMBIN}$, LTA_{ADP}, and MA_{ADP} were associated with a 3.8 (95% CI 2.1-7.0), 4.8 (95% CI 2.4 – 9.6) and 10.3 (95% CI 5.4 – 20.0) -fold relative risk of ischemic event occurrence, respectively ($p<0.0001$ for each). The occurrence of cardiac events over time is depicted in Kaplan-Meier event-time curves (Figure 2) and demonstrate the association of events to platelet function parameters by cut point ($MA_{ADP} > 47$mm and ≤ 47mm, $MA_{THROMBIN} >69$mm and ≤ 69mm, and $LTA_{ADP} >34\%$ and $\leq 34\%$). The separation of the two curves occurred early,

particularly for MA_{ADP}. Patients with high platelet function values had a continual increase in ischemic event occurrence over time. In a multivariate Cox proportional hazards model used to identify independent predictors of long term ischemic events, platelet function parameters ($MA_{THROMBIN}$, LTA_{ADP}, and MA_{ADP}) as well as patient and procedural characteristics with a $p < 0.10$ in the univariate analysis were examined (see Tables 1 and 2). When adjusted for patient and procedural covariates, MA_{ADP} was independently and significantly associated with adverse ischemic events, with a hazard ratio of 10.9 (95% CI 5.6-21.3) (Table 5). With the exception of $MA_{THROMBIN}$ and calcium channel blockers, all other variables were excluded from the model when MA_{ADP} was the platelet function parameter studied.

Quartile analysis of the platelet function parameters consistently showed low incidence of ischemic events in the lower quartiles with increasing incidence of ischemic events in higher quartiles of $MA_{THROMBIN}$ MA_{ADP} and LTA_{ADP} (Figure 5). In the fourth quartile of $MA_{THROMBIN}$ ($MA_{THROMBIN} > 72$), 43% had ischemic events (Figure 3). Of patients without an ischemic event in the highest $MA_{THROMBIN}$ quartile, 86% had a MA_{ADP} equal to or below the cutpoint of 47 mm. In contrast, 81% of patients with an ischemic event had an MA_{ADP} above this cutpoint (Figure 4.)

Bleeding Events

Fifteen patients experienced a bleeding event; 5 patients had major bleeding and 10 patients had minor bleeding. Eight of the bleeding events occurred during the index hospitalization, and 6 of these patients received a GPIIb/IIIa inhibitor. Four patients had a bleeding event within 30 days after discharge and the remaining 3 patients experienced bleeding between 30 days and 3 years follow-up. In those patients not on GPIIb/IIIa inhibitors there was no difference in any platelet function parameters (data not shown).

Of the six patients treated with a GPIIb/IIIa inhibitor who had a major bleeding event, all had an MA_{ADP} value within the first quartile at the time of the bleed. Those patients receiving the same GPIIb/IIIa inhibitor treatment (n=115) who had an MA_{ADP} value beyond the first quartile did not bleed. This indicates that the strength of the clot as measured by MA_{ADP}, and not the mere presence of a GPIIb/IIIa inhibitor, determines the probability of patient bleeding.

Although the low number of bleeding patients precludes definitive statistical analysis, nevertheless, the data trends warrant consideration. As expected, MA_{ADP} measured at or close to the time of the bleed was low, with 83% falling within the first quartile of MA_{ADP}. Notably, in the case of major bleeding, all instances fell within the first quartile. In addition, ROC curve analysis for bleeding produced a cutpoint of 31, which approximates the upper limit of the first quartile (Figure 3).

DISCUSSION

Thrombus formation is a dynamic and nonlinear process involving many interacting elements, most notably the vascular wall, blood components, and blood flow. LTA has long been the methodology of choice to determine platelet reactivity. However, our study suggests that the TEG MA parameters, MA_{ADP} and $MA_{THROMBIN}$, provide additional information for post-PCI risk assessment. *In vivo*, the strength of the clot determines whether it will resist the shear forces of the circulating blood, or impede blood flow and result in an ischemic event. The TEG MA parameters measure the strength of a clot as a direct function of the maximum dynamic properties of fibrin-platelet binding via GPIIb/IIIa and the platelet contractile system (secondary aggregation). Therefore, the TEG MA parameters may better estimate the *in vivo* situation as compared to LTA, which measures platelet aggregation mediated by fibrinogen (primary aggregation) and ignores the important contribution of platelet-fibrin interactions to both thrombosis and hemorrhage.

The existence of a high level of individual variability in platelet responsiveness to antiplatelet therapy and on-treatment platelet reactivity have been confirmed in the majority of clinical studies examining antiplatelet therapy efficacy in PCI patients.[3] Interindividual variability has also been demonstrated in thrombin-generating ability of blood.[12] It is widely accepted that ischemic events, particularly post-PCI, are multifactorial processes influenced by clinical, demographic, procedural, and hemostatic components that culminate in a thrombotic expression, i.e., an ischemic event. This study collected information on many of the accepted risk factors, and examined in particular several of the hemostatic components that contribute to and quantify the subjects' prothrombotic state. We were able to identify a set of hemostasis markers by TEG and LTA that were able to risk stratify patients for ischemic events both independently and together. In contrast, we found that few of the standard risk indicators were significantly different between patients with and without ischemic events, and were eliminated in multivariate Cox proportional hazards model analysis in the presence of platelet function measurements. These results indicate that in predicting ischemic events, the incremental contributions of most other variables besides MA_{ADP}, $MA_{THROMBIN}$, and LTA_{ADP} are statistically not significant. This was not unexpected, since standard risk factors may predispose a patient for an ischemic event, but it is the patient's hemostasis state that will ultimately determine whether the forming clot can resist the shear forces of the circulating blood and cause the event.

We showed in the PREPARE POST-STENTING study that $MA_{THROMBIN}$ and LTA_{ADP} identified patients at high risk for 6 month post-PCI ischemic event occurrence.[5] High $MA_{THROMBIN}$ (>72mm) alone was highly predictive of a recurrent ischemic event. In a study of patients undergoing a variety of non-cardiac surgical procedures, $MA_{THROMBIN}$ was also highly predictive of post-operative ischemic events.[13] In our current study of 3-year ischemic event occurrence, MA_{ADP}, $MA_{THROMBIN}$, and LTA_{ADP} were all associated with high negative predictive values, with MA_{ADP} having the highest specificity (85%, Figure 1).

In the absence of platelet inhibitors, platelets circulating in the vascular system function at maximum potential in any given patient. Maximal platelet reactivity is measured by $MA_{THROMBIN}$. However, in the presence of platelet inhibitors, circulating platelets are variably inhibited. The MA_{ADP} and MA_{AA} indicate the level of platelet reactivity to ADP and COX-1 activity, respectively. The quartile analysis of both $MA_{THROMBIN}$ and MA_{ADP} (Figure 3) indicate that patients falling into higher quartiles may require more aggressive antiplatelet therapy to prevent ischemic events, since the incidence of ischemic events increases in each quartile.

Our results indicate that in the presence of platelet ADP inhibition MA_{ADP} is a strong predictor of long-term post-PCI ischemic events regardless of how high the level of $MA_{THROMBIN}$ may be (Figure 4), suggesting its potential superiority over LTA_{ADP} and $MA_{THROMBIN}$ as a risk assessment tool. The latter also suggests its potential utility to determine individualized therapy. Eighty-seven percent of ischemic events occurred in the third and fourth quartiles of MA_{ADP} (Figure 3), which correlates well with the ROC cutpoint. Consequently, we propose the MA_{ADP} cutpoint (47mm) as the upper limit of a therapeutic target for this population. A lower limit of MA_{ADP} to define a potential therapeutic window will require a much larger study, though the presence of bleeding in the lowest quartile and the absence of bleeding in higher quartiles suggests a lower therapeutic limit of approximately 30mm, which delimits the first quartile of MA_{ADP} and corresponds to the ROC cutpoint for the MA_{ADP} taken at the time of the bleed (Figure 4). Thus, for the first time, using the bleeding cutpoint of 31 and the ischemic cutpoint of 47, a therapeutic range of 31 to 47 for MA_{ADP} can be proposed, providing maximum efficacy and safety. This is in contrast to the results of the prospective POPULAR study which demonstrated that the LTA, VerifyNow P2Y12, and Plateletworks platelet

function tests were also able to identify patients at higher risk for ischemic events, but none of the tests were able to identify patients at risk for TIMI major and minor bleeding.[14]

$MA_{THROMBIN}$ reflects the patient's maximal potential platelet reactivity and MA_{ADP} reflects platelet reactivity to ADP and assesses the individual patient's response to antiplatelet therapy. MA_{ADP} is partially determined by $MA_{THROMBIN}$ since $MA_{THROMBIN}$ represents the upper boundary of platelet reactivity. Therefore, if $MA_{THROMBIN}$ is low or normal, MA_{ADP} must also be low or normal. Thus, when $MA_{THROMBIN}$ is low or normal, the administration of a potent $P2Y_{12}$ inhibitor may enhance bleeding risk. On the other hand, if $MA_{THROMBIN}$ is high, given the wide response variability to clopidogrel, MA_{ADP} may be high, normal, or low. The analysis of patients in the fourth quartile of $MA_{THROMBIN}$ with respect to MA_{ADP} suggests the important role of platelet reactivity to ADP in determining ischemic event occurrence in these patients (Figure 4).

With this in mind, when considering the ACC/AHA PCI Guidelines for dosing and duration of post-stenting antiplatelet therapy, two areas of concern arise. First, the notion that a “one size fits all” dosing of antiplatelet drugs is the appropriate treatment for a given PCI patient is flawed. The inter-individual variability in on-treatment platelet reactivity during clopidogrel treatment confirmed by multiple prior studies predominantly utilizing LTA is now confirmed by another measurement, MA_{ADP}. Second, the implication of the Guidelines is that hemostasis is “time dependent” and that discontinuing therapy based on a fixed elapsed time after intervention, is inappropriate. Our results demonstrate that a range of maximal platelet-fibrin clot strength exists, and that 50% of patients (two highest quartiles for $MA_{THROMBIN}$) are at high risk for post-stenting ischemic events. Thus, when $P2Y_{12}$ blocking therapy is discontinued in this group, a high event rate would also be expected (Figure 3 [$MA_{THROMBIN}$ quartiles]). Therefore, our data suggest that investigations designed to assess the safety of $P2Y_{12}$ inhibitor therapy cessation should also focus on an individual assessment of hemostasis rather than only a uniform elapsed time interval post-intervention. A measurement of native maximal platelet function such as $MA_{THROMBIN}$, which is uninfluenced by ongoing antiplatelet therapy, is needed for future studies of risk assessment to determine if and when a patient may be safely taken off antiplatelet therapy following stenting.

Limitations

This was an observational study. A prospective study in which patients are treated according to the TEG results is needed to confirm that personalized management of platelet reactivity with antiplatelet drugs is more effective than conventional therapy in reducing the incidence of ischemic events and bleeding.

Conclusions

Management of patients using universal fixed-dosing dual antiplatelet therapy is associated with an unacceptable rate of recurrent events. This study was able to characterize post-PCI patients according to risk for long-term recurrence of ischemic events based on selected TEG PlateletMapping results, specifically MA_{ADP} and $MA_{THROMBIN}$. This is the first study to compare the prognostic utility of conventional aggregometry with TEG for long-term events, and also the first to examine the role of MA_{ADP} as a prognostic tool. TEG measures secondary aggregation, whereas all other point-of-care tests and LTA measure primary aggregation, ignoring the contribution of fibrin and platelet contractility in the method. This may explain the increased ability of the TEG to risk stratify. An assessment of platelet-fibrin interactions by TEG, particularly by measurement of MA_{ADP}, may facilitate future investigations of personalized antiplatelet treatment designed to reduce post-stenting ischemic events, manage bleeding risk, and determine appropriate cessation of therapy.

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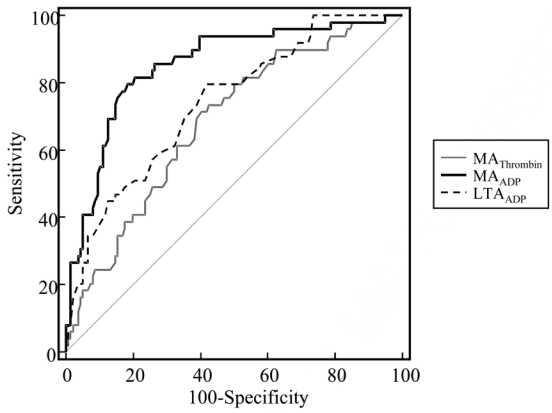
REFERENCES

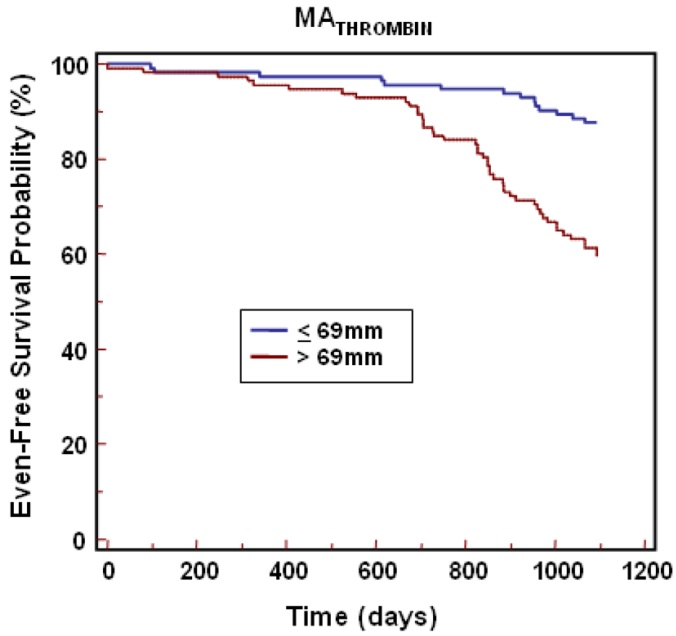
1. Furie B, Furie BC. Thrombus formation in vivo. J Clin Invest 2005;115:3355–62. [PubMed: 16322780]
2. Lee KW, Lip GY. Acute coronary syndromes: Virchow's triad revisited. BloodCoagul Fibrinolysis 2003;14:605–25.
3. Gurbel PA, Becker RC, Mann KG, et al. Platelet function monitoring in patients with coronary artery disease. J Am Coll Cardiol 2007;50:1822–34. [PubMed: 17980247]
4. Gurbel PA, Antonino MJ, Tantry US. Antiplatelet treatment of cardiovascular disease: a translational research perspective. Pol Arch Med Wewn 2008;118:289–97. [PubMed: 18619179]
5. Gurbel PA, Bliden KP, Guyer K, et al. Platelet reactivity in patients and recurrent events post-stenting: results of the PREPARE POST-STENTING Study. J Am Coll Cardiol 2005;46:1820–6. [PubMed: 16286165]
6. Bliden KP, DiChiara J, Tantry US, et al. Increased risk in patients with high platelet aggregation receiving chronic clopidogrel therapy undergoing percutaneous coronary intervention: is the current antiplatelet therapy adequate? J Am Coll Cardiol 2007;49:657–66. [PubMed: 17291930]
7. Thygesen K, Alpert JS, White HD. Joint ESC/ACCF/AHA/WHF Task Force for the Redefinition of Myocardial Infarction. Universal definition of myocardial infarction. Eur Heart J 2007;28:2525–38. [PubMed: 17951287]
8. Mauri L, Hsieh WH, Massaro JM, et al. Stent thrombosis in randomized clinical trials of drug-eluting stents. N Engl J Med 2007;356:1020–9. [PubMed: 17296821]
9. Antman EM, Cohen M, Bernink PJ, et al. The TIMI risk score for unstable angina/non-ST elevation MI: A method for prognostication and therapeutic decision making. JAMA 2000;284:835–42. [PubMed: 10938172]
10. Gurbel PA, Tantry US. The relationship of platelet reactivity to the occurrence of post-stenting ischemic events: emergence of a new cardiovascular risk factor. Rev Cardiovasc Med 2006;7(Suppl 4):S20–8. [PubMed: 17224887]
11. Gurbel PA, Antonino MJ, Bliden KP, et al. Platelet reactivity to adenosine diphosphate and long-term ischemic event occurrence following percutaneous coronary intervention: a potential antiplatelet therapeutic target. Platelets 2008;19:595–604. [PubMed: 19012177]
12. Gurbel PA, Bliden KP, Guyer K, et al. Delayed thrombin-induced platelet-fibrin clot generation by clopidogrel: a new dose-related effect demonstrated by thrombelastography in patients undergoing coronary artery stenting. Thromb Res 2007;119:563–70. [PubMed: 16806409]
13. McCrath DJ, Cerboni E, Frumento RJ, et al. Thromboelastography maximum amplitude predicts postoperative thrombotic complications including myocardial infarction. Anesth Analg 2005;100:1576–83. [PubMed: 15920177]
14. Breet NJ, van Werkum JW, Bouman HJ, et al. Comparison of platelet function tests in predicting clinical outcome in patients undergoing coronary stent implantation. JAMA 2010;303:754–62. [PubMed: 20179285]

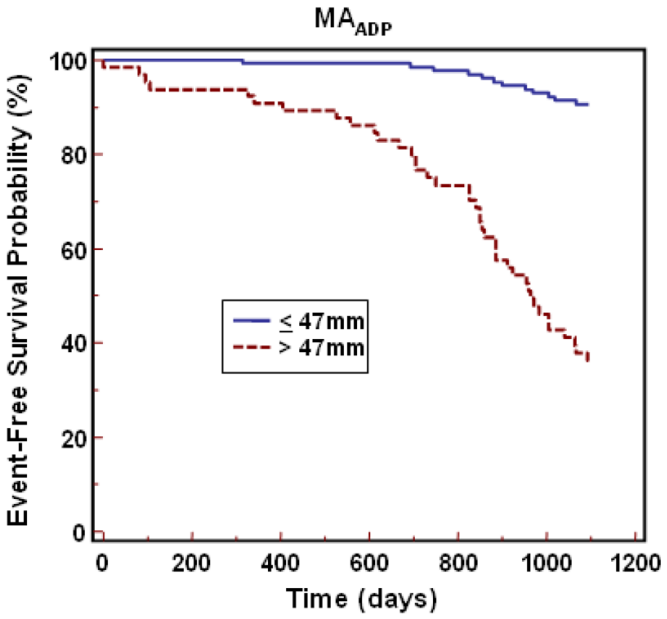


	Cutpoint	Sensitivity (%)	Specificity (%)	AUC (95% CI)	PPV (%)	NPV (%)
MA_{ADP}	>47mm	76	85	0.84 (0.78-0.89)*	64	91
LTA_{ADP}	>34%	80	59	0.75 (0.68-0.80)*	41	89
$MA_{THROMBIN}$	>69mm	76	60	0.70 (0.64-0.76)*	40	88

AUC = Area Under the Curve, PPV = Positive Predictive Value, NPV = Negative Predictive Value
* p< 0.0001

Figure 1.
Comparison of receiver operating characteristic curves for $MA_{THROMBIN}$, MA_{ADP}, and LTA_{ADP}.





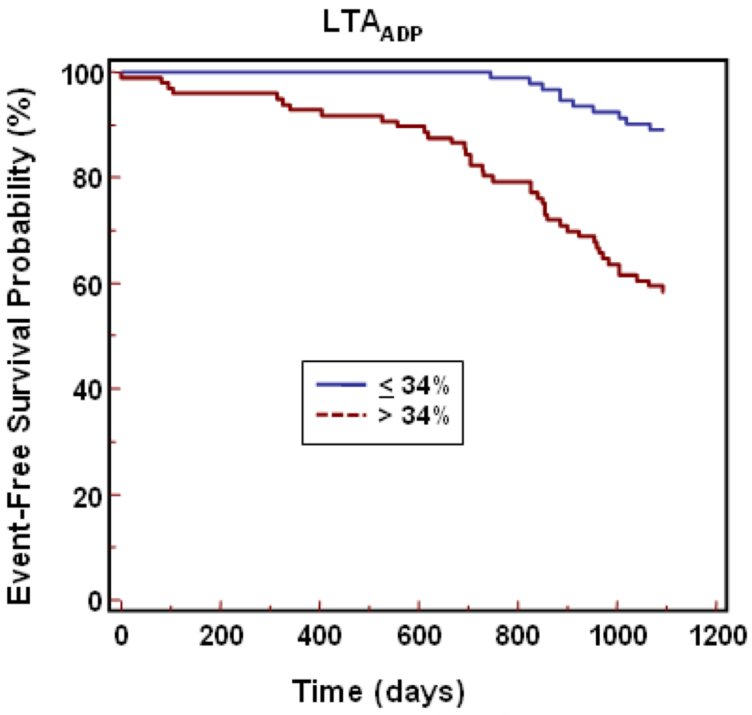
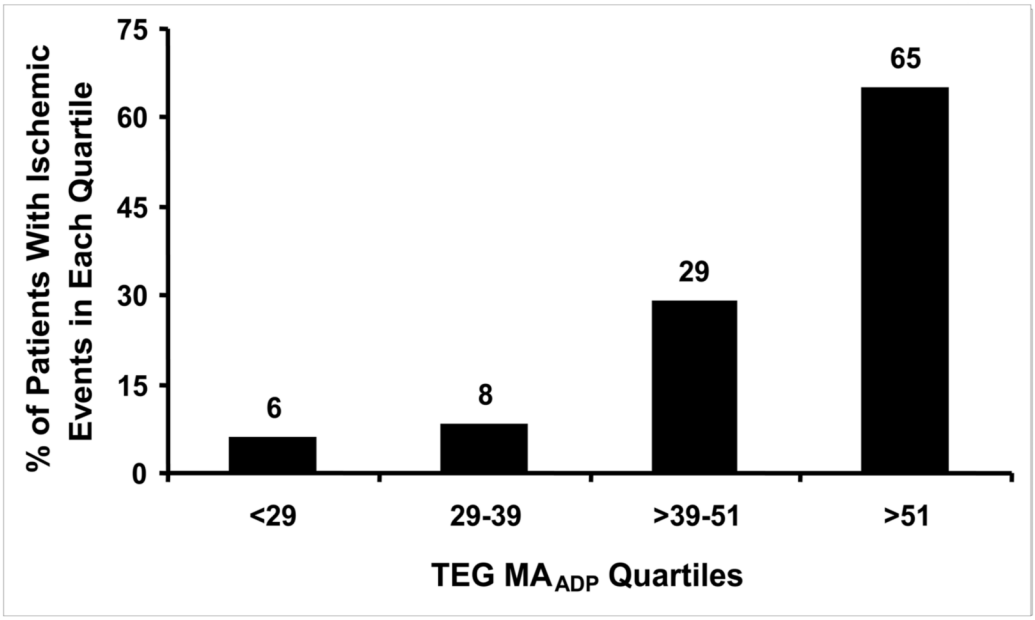
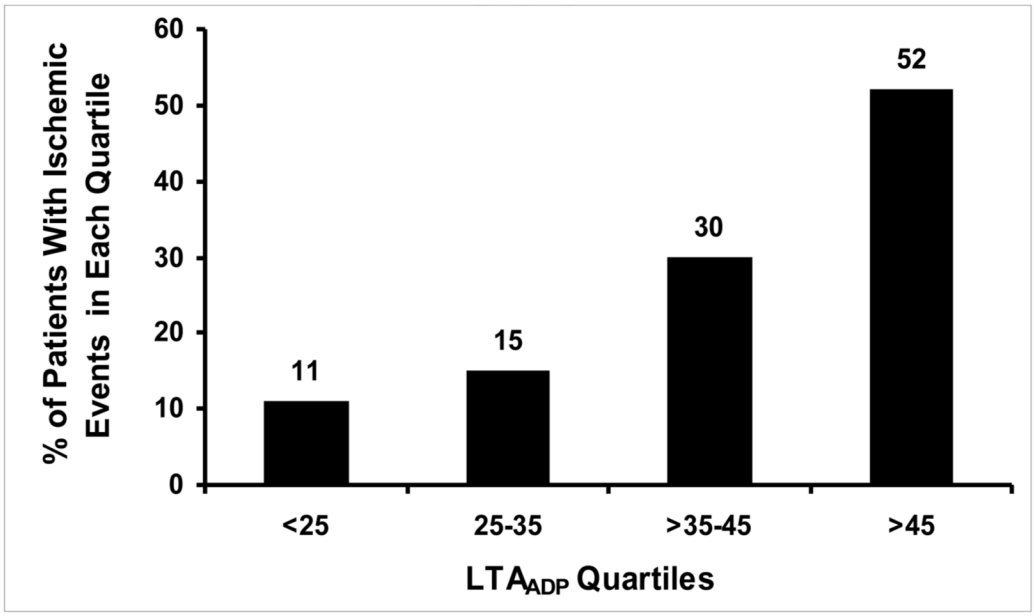


Figure 2.
Cumulative incidence of first ischemic events (Kaplan Meier) during 3-year follow-up period for platelet function parameters.





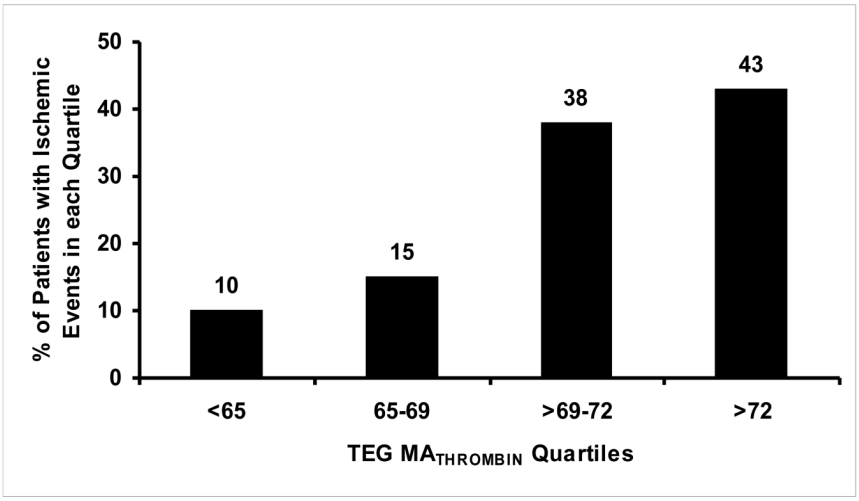


Figure 3.
Quartile distribution of ischemic events for each platelet function parameter.

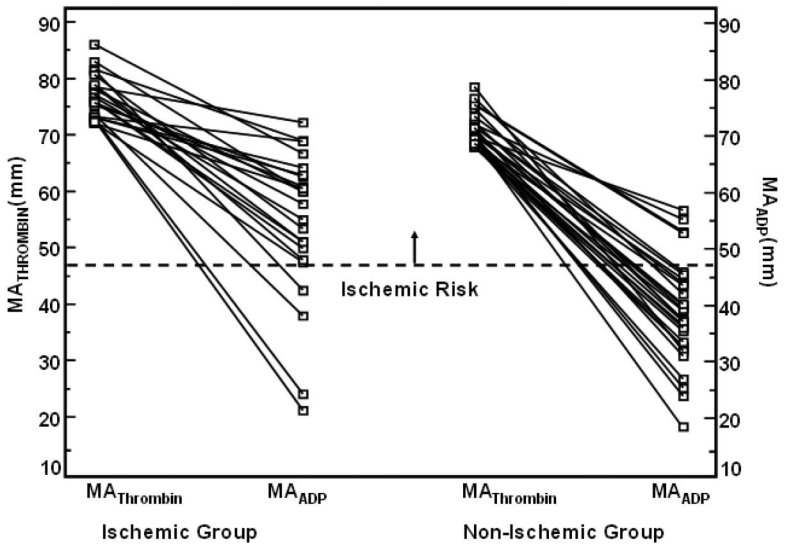


Figure 4.

Fourth quartile of $MA_{THROMBIN}$ showing $MA_{THROMBIN}$ with corresponding MA_{ADP} value for each patient. Left portion displays patients with repeat ischemic events and right portion without ischemic events. Dotted lines indicate cutpoints for ischemic and bleeding risk, and delineate a possible therapeutic range.

Table 1

Patient Characteristics

	Total Group (n=225)	Ischemic Group (n=59)	Non-Ischemic Group (n=166)	p-value
Age (years)	66 ±12	66 ±12	64 ± 11	0.24
Gender and Ethnicity n, (%)				
Caucasian male	121 (54)	30 (48)	91 (55)	0.18
Caucasian female	44 (20)	14 (22)	30 (18)	0.25
African American male	30 (13)	8 (13)	22 (13)	1.0
African American female	30 (13)	7 (11)	23 (14)	0.28
BMI (kg/m^2)	29 ± 7	30 ± 6	29 ± 7	0.30
Systolic BP (mmHg)	144 ± 25	144 ± 21	146 ± 24	0.57
Diastolic BP (mmHg)	75 ± 17	75 ± 16	76 ± 17	0.69
Presentation n, (%)				
Elective	159 (71)	44 (75)	115 (69)	0.19
Unstable Angina	50 (22)	12 (20)	38 (22)	0.37
Myocardial Infarction	16 (7)	3 (5)	13 (8)	0.22
Risk Factors/Past Medical History n, (%)				
Smoking	124 (55)	31 (52)	93 (56)	0.29
Family history of CAD	106 (48)	31 (52)	75 (47)	0.25
Hypertension	167 (74)	47 (79)	118 (72)	0.25
Hyperlipidemia	179 (80)	52 (89)	127 (77)	0.02
Diabetes	87 (41)	33 (56)	60 (36)	0.004
Peripheral Vascular Disease	19 (8)	6 (10)	13 (8)	0.32
Prior Myocardial Infarction	75 (33)	22 (37)	53 (32)	0.24
Prior CABG	54 (24)	14 (23)	40 (24)	0.44
Prior PTCA	79 (35)	27 (47)	52 (31)	0.01
Prior CVA	27 (12)	5 (9)	22 (13)	0.21
Baseline Medications n, (%)				
Beta blockers	190 (84)	49 (83)	141 (85)	0.36
ACE inhibitors	146 (65)	38 (65)	108 (65)	1.0
Calcium channel blockers	59 (26)	23 (39)	36 (22)	0.005
Lipid lowering agents	184 (82)	50 (85)	134 (81)	0.45
PPI	76 (34)	21 (36)	55 (33)	0.34
Laboratory Data				
WBC (× $1000/mm^3$)	7.8 ± 3.0	8.6 ± 3.8	7.5 ± 2.4	0.12
Platelets (× $1000/mm^3$)	231 ± 71	239 ± 69	228 ± 70	0.29
Hemoglobin (g/dL)	13.4 ± 2.0	13.0 ± 1.9	13.5 ± 2.1	0.10
Hematocrit (%)	39.9 ± 5.2	39.4 ± 5.0	40.1 ± 5.3	0.37

	Total Group (n=225)	Ischemic Group (n=59)	Non-Ischemic Group (n=166)	p-value
Creatinine (g/dL)	1.1 ± 0.5	1.1 ± 0.5	1.1 ± 0.6	1.0

ACE=angiotensin converting enzyme; BMI=body mass index; CABG=coronary artery bypass graft surgery; CAD=coronary artery disease; CVA=cerebrovascular accident; PTCA=percutaneous transluminal coronary angioplasty; WBC=white blood cells: PPI=proton pump inhibitors.

Table 2

Procedural Characteristics

	Total Group (n=225)	Ischemic Group (n=59)	Non-Ischemic Group (n=166)	p-value
Length of procedure (min.)	60 ± 32	56 ± 27	59 ± 33	0.52
Ejection fraction (%)	53 ± 38	49 ± 11	55 ± 15	0.005
Number of vessels treated	1.3 ± 0.6	1.4 ± 0.6	1.3 ± 0.5	0.21
Culprit Lesion Morphology n, (%)				
De novo	200 (88)	50 (85)	150 (90)	0.15
Restenotic	25 (12)	9 (15)	16 (10)	0.15
Bifurcation lesion, Severe Calcification, Evidence of Thrombus, or Total Occlusion	22 (10)	10 (17)	12 (7)	0.01
ACC/AHA Lesion Score n, (%)				
Type A	19 (8)	4 (7)	15 (9)	0.32
Type B1	65 (29)	13 (22)	52 (31)	0.10
Type B2	51 (23)	13 (22)	38 (23)	0.44
Type C	90 (40)	29 (49)	61 (37)	0.05
Culprit Lesion Location n, (%)				
LAD	77 (34)	19 (32)	58 (35)	0.34
CX	56 (25)	11 (19)	45 (27)	0.11
RCA	80 (36)	26 (44)	54 (32)	0.05
SVG	12 (5)	3 (5)	9 (6)	0.39
Stent Types n, (%)				
Drug eluting	169 (75)	49 (78)	120 (72)	0.19
Bare metal	51 (23)	11 (25)	40 (24)	0.44
PTCA only	9 (4)	3 (5)	6 (4)	0.37
Reference vessel diameter (mm)	3.0 ± 0.5	2.9 ± 0.4	3.1 ± 0.5	0.005
Total lesion length (mm)	22 ± 14	21 ± 9	22 ± 16	0.64
Pre-Dilation n, (%)	121 (54)	30 (51)	91 (55)	0.29
Post Dilation n, (%)	109(48)	32 (54)	77 (46)	0.15
Total Stents per patient, n	1.5 ± 1.0	1.7 ± 1.2	1.4 ± 0.8	0.03
Procedural Success n, (%)	219	58 (98)	161 (97)	0.34

Cx=circumflex artery; LAD=left anterior descending artery; PTCA=percutaneous transluminal coronary angioplasty; RCA=right coronary artery; SVG=saphenous vein graft ACC/AHA = American College of Cardiology/American Heart Association.

Table 3

First Ischemic Events

	Total Group (n=225)		
	Month < 1	**Month 1-36**	**Month 0-36**
Ischemic Events, n (%)			
Cardiac Death	1 (0.4)	0 (0.0)	1 (0.4)
Stent Thrombosis	4 (1.7)	2 (0.8)	6 (2.5)
Myocardial Infarction	0 (0.0)	17 (7.5)	17 (7.5)
Target Vessel Revascularization	0 (0.0)	26 (11.6)	26 (11.6)
Non-Target Vessel Revascularization	0 (0.0)	9 (4.0)	9 (4.0)
Total Ischemic Events, n (%)	**5 (2.2)**	**54 (24.0)**	**59 (26.2)**

Table 4

Platelet Function Parameters

	Ischemic Group	Non-Ischemic Group	p-value
Thrombelastography (TEG)			
$MA_{THROMBIN}$ (mm)	72±6	67±6	<0.0001
MA_{ADP} (mm)	51 ± 12	35±13	<0.0001
MA_{AA} (mm)	18 ± 10	15 ± 10	0.04
Light Transmittance Aggregation (LTA)			
LTA_{ADP} (aggregation, %)	45 ± 13	31±15.	<0.0001

ADP = Adenosine diphosphate, AA = Arachidonic acid

Table 5

Cox-Proportional Hazards Regression

Covariate	Adjusted HR (95% CI)	P Value
MA_{ADP} >47mm	10.9 (5.6 to 21.3)	<0.0001
$MA_{THROMBIN}$ >69 mm	3.5 (1.9 to 6.4)	<0.0001
Calcium channel blockers	2.3 (1.2 to 4.1)	0.008
LTA_{ADP} >34%	5.6 (2.7 to 11.6)	<0.0001
Hx of prior PTCA	2.1 (1.2 to 3.7)	0.01
Calcium channel blockers	2.2 (1.2 to 4.3)	0.016

Each cluster represents analysis of each of the platelet function parameters and covariates (only variables retained by the analysis are shown).